

**ABSTRACT**

5 The present invention relates to the use of site-specific  
nucleic acid nicking enzymes to create single-stranded regions  
in duplex nucleic acids. Such single-stranded regions can take  
the form of gaps interior to the duplex, or terminal single-  
stranded regions. Single-stranded termini can be crafted to  
allow linkage of various elements *via* base-pairing with  
10 elements containing a complementary single-stranded region.  
This joining is useful, for example, in an ordered, oriented  
assembly of DNA modules to create cloning or expression  
vectors. This joining is also useful in attaching detection  
probes and purifying DNA molecules containing the single-  
15 stranded region. Gaps are useful in similar applications,  
including attaching detection or purification probes.